

## Physiological Study About Imidacloprid Toxicity And The Role Of Vitamin "C" As A Protective Agent On Japanese Quails

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### Abstract

**Background:** Imidacloprid belongs to the group of nitroguanidines. Many representatives of this class of compounds and imidacloprid in particular , have an excellent insecticide action. Among the various hazards associated with insecticide use is the possibility of its biological accumulation which produces real problems, considering that many animal tissues and milk are ingested by human beings, which may cause clinical and subclinical effects leading to losses in animal performance or in residue contamination of animal products which may later be consumed by humans. Thus, the disappearance of insecticide residues at a given location does not mean the end of the problem, but it might be translocated, bioconcentrated or converted into more dangerous compounds.

The present study was designed to evaluate toxic effects of imidacloprid insecticide and possible ameliorating role of vitamin C on Japanese quails.

**Materials & Methods:** The tested quails divided into four groups , the first group served as control , the second group treated with vitamin C only , the third group treated with imidacloprid singly and the fourth group treated with imidacloprid combined with vitamin C for 3 and 6 weeks of treatment and 3 weeks of recovery periods. Serum biochemical parameters were measured , blood glucose level , cholinesterase (AChE), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities , total protein , albumin , globulin , A/G ratio , total lipid and total cholesterol . In the same time ALT , AST , total protein , total lipid and total cholesterol were measured in liver , kidney and brain tissues.

**Results:** The data obtained revealed insignificant changes after quails treated with vitamin C singly in all biochemical parameters ( serum and tissues ) throughout the experimental periods . Highly significant increases were observed in serum glucose level , LDH , ALT , AST , ALP activities total lipid and cholesterol in imidacloprid treated group during the experimental period and after imidacloprid combined with vitamin C. These increases were observed after 3 and 6 weeks of treatment. In the same time , significant inhibition in cholinesterase activity in imidacloprid treated group with or without vitamin C was detected.

Furthermore , significant decrease were observed in serum total protein , albumin and globulin of groups treated with imidacloprid only, whereas a marked amelioration was detected in these parameters in addition to A/G ratios in quails treated with imidacloprid + vitamin C throughout the experimental periods.

Creatinine revealed significant increases in imidacloprid treated group during the experimental periods . No alterations were recorded in creatinine concentration of groups treated with both imidacloprid and vitamin C specially after 6weeks . Serum uric acid recorded insignificant changes in all treated groups.

However , highly significant increases were recorded in ALT and AST activities in liver and kidney after treatment with imidacloprid alone or combined with vitamin C till the end of the experiment . In addition , significant decreases were observed in liver and kidney total lipids and in liver total cholesterol after treatment with imidacloprid throughout the experimental

periods. Also, significant decrease were observed in liver total protein after 3 and 6 weeks of treatment with imidacloprid alone or combined with vitamin C.

**In conclusion:** According to the previous results, we conclude that vitamin C may reduce the toxicity of imidacloprid, but this protection may require higher doses or more time for recovery.

**Key words :** Imidacloprid, Vitamin C, Antioxidant, Biochemical Parameters, Serum Liver, Kidney, Brain, Japanese quails.

## Introduction

Adverse effects of pesticides on wild life are usually recognized only after field application. Most toxicological investigations with game birds have been undertaken in retrospect, after the product has appeared on the market. Even today, the development of a new insecticide does not normally include a direct evaluation of its hazard to wild life, but it is neither feasible nor practical to study the effect new product in all such game species. Imidacloprid is a new insecticidal for seed treatment and it belongs to a new active group nitroguanidine (Tomizawa and Casida, 2005). It is a widely used synthetic insecticide, it is a nicotinic acetylcholine receptor agonist (Matsuda et al., 2001). This chemical works by interfering with the transmission of stimuli in the insect nervous system. It causes a blockage in a type of neuronal pathway of insects.

This blockage leads to the accumulation of acetylcholine, resulting in the insect's paralysis, and eventually death (Karsbay and Oguz, 2005). Insecticides have been observed to accentuate oxidative stress by generation of free radicals in rat tissues, these free radicals play an important role in toxicity of pesticides and environmental chemicals, by diminishing the antioxidants or altering oxygen free radicals scavenging enzyme system (Banerjee et al., 1999 and Kamboj et al., 2006).

Vitamin C is hydrophilic and most important free radical scavenger in extracellular fluids, trapping radicals in the aqueous phase, and protecting biomembranes from peroxidative damage (Sulak et al., 2005 and Uzunhisarcikli et al., 2007).

The aim of this study was to evaluate the toxicity of imidacloprid insecticide and protective effect of vitamin C in Japanese quails (*Coturnix coturnix japonica*) at the

end of at 3, 6 weeks of treatment and recovery period (3 weeks).

## Material And Methods

### Chemicals :

- Imidacloprid.

common name : imidacloprid (BSI, draft E-ISO).

chemical name : 1 - [ ( 6 - chloro - 3 - pyridinyl ) methyl ] - N - nitro - 2 - imidazolidinimine .

LD50 of imidacloprid was determined according to the equation of Behren and Karber (1953) as follows :

$$LD50 = Dm - \frac{S(Z-d)}{m}$$

Where :

m

DM = the higher dose used.

Z = the number of dead animals of two successive doses divided by two.

d = the difference between two successive doses.

S = Total sum of ( Z . d ).

m = the number of birds in each group.

The LD50 was determined to be 31 mg/kg b.wt. for imidacloprid insecticide in Japanese quails.

### Animals

Sixty mature male Japanese quails (*Coturnix coturnix japonica*) weighing 130 - 150 g. were obtained from the poultry research farm, Faculty of Agriculture, Al-Azhar University. The birds were kept under normal laboratory conditions, and were divided into 4 groups (15 birds each) in separate cages throughout the study period, the first group served as a control group, the second group injected with vitamin C (0.08 mg/kg body weight), the third group was treated orally with a dose equals to the 1/50 LD50 of imidacloprid

insecticide via stomach tube and the fourth group was treated with the same doses of vitamin C and imidacloprid conjointly. After 3 weeks of treatment, 5 birds were chosen randomly from each group and sacrificed. The same treatments continued for the rest of the birds and after additional three weeks, 5 birds were taken out from each group and sacrificed. The rest of the birds were left without any treatment for further 3 weeks as a recovery period after which the last batch of birds was killed to assess the impact of recovery.

#### Collection of serum :

Blood samples were collected in glass centrifuge tubes and left for clot formation, then kept for few minutes in refrigerator. Sera were separated by centrifugation at 3000 rpm for 15 minutes and kept at  $-20^{\circ}\text{C}$  till used for biochemical analysis.

#### Preparation of tissues for biochemical analysis :

After each bird was sacrificed it was rapidly dissected and some organs were selected ( liver, kidney and brain ). Pieces of each organ were cleaned by dist. water and weighted, then put in an appropriate amount of 30% potassium hydroxide for determination of total protein & concentrated sulphuric acid for determination of total lipid and in saline solution for determination ALT, AST activities and total cholesterol.

#### Biochemical Analysis :

A portion of the clear supernatant serum was used immediately for glucose determination according to the enzymatic colorimetric method described by *Trinder (1969)*. Serum cholinesterase activity was determined calorimetrically according to *Ellman et al. (1961)*. Determination of serum lactate dehydrogenase (LDH) was carried out according to the method of *Wroblewski and La Dua (1995)*. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were estimated according to the method described by *Reitman and Frankel (1957)*. Serum alkaline phosphatase (ALP) was determined by the method of *Belfield and Goldberg (1971)*. The contents of total cholesterol and total lipids were assayed

according to *Allain et al. (1974)* and *Knight et al. (1972)*, respectively. Total protein and albumin levels were estimated according to the methods described by *Doumas (1975)* and *Doumas (1971)*, respectively. Serum globulin was calculated according to *Lanter (1975)*. Serum contents of uric acid and creatinine were estimated according to the methods described by *Steven (1970)* and *Husdan & Rapoport (1968)*, respectively.

#### Statistical analysis of the data :

In the present work, the data are presented in tables as ( mean  $\pm$  standard error ). The significance of difference between the means was calculated using the student "t-" test according to the method of *Snedecor and Cochran (1980)*.

#### Results

The represented data showed insignificant changes after quails treated with vitamin C in all biochemical parameters throughout the experimental periods. The data represented in table 1 display the effect of treatment with imidacloprid and /or vitamin C on serum glucose, LDH, Cholinesterase, ALT, AST and ALP activities of Japanese quails.

A highly significant increase ( $P < 0.01$ ) in glucose, LDH, ALP, AST and ALT was detected in all groups with imidacloprid alone or treated in combination with vitamin C. A partial amelioration was noted in LDH, ALP, ALT after a recovery period in groups treated with imidacloprid plus vitamin C in comparison with the corresponding control groups. On the other hand, serum cholinesterase activity was significantly inhibited ( $P < 0.01$ ) in all experimental groups.

Results presented in table 2 indicate that there were a marked significant increases in serum total lipids and total cholesterol in all treated groups. In the same table the data revealed highly significant decreases in serum total protein and albumin during the experimental periods after the Japanese quails treated with imidacloprid, however treating birds with imidacloprid combined with vitamin C showed amelioration changes, they revealed

insignificant alterations in levels of total protein, albumin and globulin. Also, the obtained data showed insignificant difference in A/G ratio in all treated groups throughout the experimental periods.

In table 3, creatinine levels revealed highly significant increase in birds treated with imidacloprid alone, while an amelioration (insignificantly) of creatinine level was detected in groups treated with imidacloprid + vitamin C specially after 6 weeks and in recovery group. Also, serum uric acid level had insignificant changes in all treated groups.

The tabulated data in table 4 recorded insignificant changes in total protein of kidney and brain tissues in all treated groups throughout the experimental periods. On the other hand, marked decrease was observed in liver tissue in imidacloprid treated groups with and without vitamin C after 3 and 6 weeks of

treatment. But it was insignificantly changed in the recovery group.

A highly significant increase was observed in ALT and AST activity of liver and kidney contents in quails treated with imidacloprid alone or when combined with vitamin C and continued till the end of the experiment (table 5), while insignificant changes were observed in brain content in all groups throughout the experimental periods as compared with control group.

A significant decrease ( $P < 0.05$ ) was observed in total lipids and cholesterol of liver tissue in groups treated with imidacloprid alone. Also, the same results were detected in total lipids of the kidney tissue. Insignificant changes were observed in the total lipids and cholesterol of brain tissue in addition to the total cholesterol in the kidney tissue of all groups throughout the experimental periods (table 6).

**Table (1): Effect of Imidacloprid and Vitamin C on serum Glucose , LDH, Cholinesterase , ALT , AST and ALP activities of Japanese quails at 3,6 weeks and recovery period (3 weeks )**

Parameters	Time Groups	3 weeks	6 weeks	Recovery (3 weeks)
Glucose (mg/dl)	Control	121.60±1.97	124.40±3.74	125.50±0.56
	Vitamin C	123.50 <sup>NS</sup> ±2.95	125.50 <sup>NS</sup> ±0.56	127.70 <sup>NS</sup> ±0.86
	Imidacloprid	165.40 <sup>**</sup> ±5.79	161.50 <sup>**</sup> ±1.94	158.10 <sup>**</sup> ±1.09
	Imidacloprid + Vitamin C	144.00 <sup>**</sup> ±3.56	141.90 <sup>**</sup> ±2.18	135.90 <sup>NS</sup> ±5.35
Cholinesterase activity (U/L)	Control	1457.00±2.17	1450.60±2.17	1448.40±0.51
	Vitamin C	1457.80 <sup>NS</sup> ±2.47	1452.00 <sup>NS</sup> ±3.13	1444.00 <sup>NS</sup> ±2.41
	Imidacloprid	1410.40 <sup>**</sup> ±1.12	1420.00 <sup>**</sup> ±2.03	1430.40 <sup>**</sup> ±0.93
	Imidacloprid + Vitamin C	1412.40 <sup>**</sup> ±1.12	1420.00 <sup>**</sup> ±2.03	1438.10 <sup>**</sup> ±0.86
LDH (U/L)	Control	787.60±1.05	798.10±3.80	790.50±2.86
	Vitamin C	790.30 <sup>NS</sup> ±0.58	800.20 <sup>NS</sup> ±2.86	800.50 <sup>NS</sup> ±2.85
	Imidacloprid	842.10 <sup>**</sup> ±1.32	863.40 <sup>**</sup> ±2.13	834.30 <sup>**</sup> ±1.76
	Imidacloprid + Vitamin C	800.20 <sup>**</sup> ±2.86	812.20 <sup>*</sup> ±2.03	806.50 <sup>NS</sup> ±2.06
ALP (U/L)	Control	126.40±0.41	127.90±0.41	128.30±0.31
	Vitamin C	126.20 <sup>NS</sup> ±0.44	127.80 <sup>NS</sup> ±0.32	127.50 <sup>NS</sup> ±0.22
	Imidacloprid	139.70 <sup>**</sup> ±0.44	136.30 <sup>**</sup> ±0.32	137.30 <sup>**</sup> ±0.19
	Imidacloprid + Vitamin C	132.80 <sup>**</sup> ±0.32	131.90 <sup>**</sup> ±0.17	129.10 <sup>NS</sup> ±0.29
AST (U/L)	Control	38.20±0.37	39.20±0.80	40.80±1.24
	Vitamin C	37.20 <sup>NS</sup> ±1.24	38.20 <sup>NS</sup> ±0.37	40.80 <sup>NS</sup> ±1.24
	Imidacloprid	46.00 <sup>**</sup> ±0.95	46.00 <sup>**</sup> ±0.95	42.20 <sup>*</sup> ±0.92
	Imidacloprid + Vitamin C	44.00 <sup>**</sup> ±0.63	42.20 <sup>*</sup> ±0.92	40.80 <sup>NS</sup> ±1.24
ALT (U/L)	Control	16.90±0.68	17.70±0.42	18.60±0.39
	Vitamin C	16.60 <sup>NS</sup> ±1.86	16.90 <sup>NS</sup> ±1.88	18.00 <sup>NS</sup> ±0.51
	Imidacloprid	29.50 <sup>**</sup> ±0.42	28.20 <sup>**</sup> ±1.12	27.20 <sup>**</sup> ±1.72
	Imidacloprid + Vitamin C	27.20 <sup>**</sup> ±1.75	24.70 <sup>**</sup> ±0.42	22.70 <sup>**</sup> ±0.42

Data represented as mean ± stander error.

NS=Non Significant.

\*=Significant(P<0.05).

\*\*=Highly Significant(P<0.01).



Table (2): Effect of Imidacloprid and Vitamin C on serum total lipid , total cholesterol , total protein , albumin , globulin and A/G ratio of Japanese quails at 3,6 weeks and recovery period (3 weeks ).

Parameters	Time Groups	3 weeks	6 weeks	Recovery (3 weeks)
Total lipid (mg/dl)	Control	202.00±3.74	222.80±2.80	217.80±2.52
	Vitamin C	202.00 <sup>NS</sup> ±3.74	224.60 <sup>NS</sup> ±2.25	222.60 <sup>NS</sup> ±1.90
	Imidacloprid	272.00 <sup>**</sup> ±3.74	279.00 <sup>**</sup> ±1.87	237.70 <sup>**</sup> ±2.52
	Imidacloprid + Vitamin C	270.00 <sup>**</sup> ±2.53	262.80 <sup>**</sup> ±2.62	237.80 <sup>**</sup> ±2.52
Total cholesterol (mg/dl)	Control	112.80±0.34	115.80±0.22	115.80±0.19
	Vitamin C	114.60 <sup>NS</sup> ±2.21	115.80 <sup>NS</sup> ±0.22	114.60 <sup>NS</sup> ±2.21
	Imidacloprid	140.10 <sup>**</sup> ±2.23	141.80 <sup>**</sup> ±2.23	135.80 <sup>**</sup> ±3.37
	Imidacloprid + Vitamin C	131.02 <sup>**</sup> ±2.28	132.70 <sup>**</sup> ±2.33	128.90 <sup>**</sup> ±3.27
Total protein (g/dl)	Control	5.50±0.13	5.70±0.09	5.90±0.24
	Vitamin C	5.50 <sup>NS</sup> ±0.35	5.80 <sup>NS</sup> ±0.09	6.02 <sup>NS</sup> ±0.29
	Imidacloprid	4.20 <sup>**</sup> ±0.07	4.40 <sup>**</sup> ±0.07	4.80 <sup>**</sup> ±0.09
	Imidacloprid + Vitamin C	5.10 <sup>NS</sup> ±0.27	5.50 <sup>NS</sup> ±0.35	6.10 <sup>NS</sup> ±0.27
Albumin (g/dl)	Control	4.10±0.09	4.10±0.06	4.30±0.07
	Vitamin C	4.10 <sup>NS</sup> ±0.08	4.10 <sup>NS</sup> ±0.05	4.20 <sup>NS</sup> ±0.04
	Imidacloprid	3.30 <sup>**</sup> ±0.08	3.40 <sup>**</sup> ±0.05	3.80 <sup>**</sup> ±0.09
	Imidacloprid + Vitamin C	3.90 <sup>NS</sup> ±0.04	4.02 <sup>NS</sup> ±0.29	4.20 <sup>NS</sup> ±0.04
Globulin (g/dl)	Control	1.40±0.15	1.74±0.09	1.64±0.27
	Vitamin C	1.46 <sup>NS</sup> ±0.30	1.64 <sup>NS</sup> ±0.15	1.82 <sup>NS</sup> ±0.31
	Imidacloprid	0.90 <sup>*</sup> ±0.15	0.80 <sup>**</sup> ±0.13	1.30 <sup>NS</sup> ±0.08
	Imidacloprid + Vitamin C	1.20 <sup>NS</sup> ±0.32	1.54 <sup>NS</sup> ±0.34	1.80 <sup>NS</sup> ±0.18
A/G ratio	Control	2.96±0.29	2.50±0.16	2.97±0.51
	Vitamin C	2.25 <sup>NS</sup> ±0.56	2.58 <sup>NS</sup> ±0.26	2.58 <sup>NS</sup> ±0.39
	Imidacloprid	4.20 <sup>NS</sup> ±0.89	5.21 <sup>NS</sup> ±1.47	2.76 <sup>NS</sup> ±0.22
	Imidacloprid + Vitamin C	3.30 <sup>NS</sup> ±0.89	2.94 <sup>NS</sup> ±0.47	2.46 <sup>NS</sup> ±0.29

Data represented as mean ± stander error.

NS=Non Significant.

\*=Significant(P<0.05).

\*\*=Highly Significant(P<0.01).

**Table (3): Effect of Imidacloprid and Vitamin C on serum creatinine(mg/dl) and serum uric acid (mg/dl) of Japanese quails at 3,6 weeks and recovery period (3 weeks ).**

Parameters	Time Groups	3 weeks	6 weeks	Recovery (3 weeks)
Serum creatinine (mg/dl)	Control	1.50±0.12	1.60±0.14	1.60±0.05
	Vitamin C	1.40 <sup>NS</sup> ±0.12	1.50 <sup>NS</sup> ±0.04	1.60 <sup>NS</sup> ±0.08
	Imidacloprid	2.70 <sup>**</sup> ±0.07	2.20 <sup>**</sup> ±0.03	1.80 <sup>*</sup> ±0.04
	Imidacloprid + Vitamin C	2.20 <sup>**</sup> ±0.04	2.10 <sup>NS</sup> ±0.19	1.90 <sup>NS</sup> ±0.15
Serum uric acid (mg/dl)	Control	10.50±0.31	11.10±0.23	11.40±0.32
	Vitamin C	11.00 <sup>NS</sup> ±0.01	11.40 <sup>NS</sup> ±0.10	11.40 <sup>NS</sup> ±0.31
	Imidacloprid	11.50 <sup>NS</sup> ±0.39	11.80 <sup>NS</sup> ±0.29	11.80 <sup>NS</sup> ±0.22
	Imidacloprid + Vitamin C	10.70 <sup>NS</sup> ±0.09	10.80 <sup>NS</sup> ±0.21	11.50 <sup>NS</sup> ±0.10

**Table (4): Effect of Imidacloprid and Vitamin C on liver , kidney and brain total protein of Japanese quails at 3,6 weeks and recovery period (3 weeks ).**

Parameters		Time Groups	3 weeks	6 weeks	Recovery (3 weeks)
Total Protein (mg / g. tissue)	Liver	Control	23.60±0.15	24.40±0.15	24.20±0.07
		Vitamin C	23.70 <sup>NS</sup> ±0.16	24.30 <sup>NS</sup> ±0.19	24.20 <sup>NS</sup> ±0.07
		Imidacloprid	20.20 <sup>**</sup> ±0.28	23.10 <sup>**</sup> ±0.21	24.10 <sup>NS</sup> ±0.09
		Imidacloprid + Vitamin C	22.80 <sup>*</sup> ±0.16	23.90 <sup>*</sup> ±0.07	24.20 <sup>NS</sup> ±0.07
	Kidney	Control	24.10±0.14	24.70±0.04	24.80±0.06
		Vitamin C	23.80 <sup>NS</sup> ±0.12	24.60 <sup>NS</sup> ±0.07	24.50 <sup>NS</sup> ±0.05
		Imidacloprid	23.90 <sup>NS</sup> ±0.03	24.50 <sup>NS</sup> ±0.80	24.60 <sup>NS</sup> ±0.07
		Imidacloprid + Vitamin C	24.00 <sup>NS</sup> ±0.07	24.50 <sup>NS</sup> ±0.08	24.80 <sup>NS</sup> ±0.05
	Brain	Control	26.00±0.57	25.70±0.23	25.70±0.23
		Vitamin C	25.00 <sup>NS</sup> ±0.08	25.20 <sup>NS</sup> ±0.09	25.50 <sup>NS</sup> ±0.07
		Imidacloprid	24.50 <sup>NS</sup> ±0.16	25.10 <sup>NS</sup> ±0.12	25.50 <sup>NS</sup> ±0.12
		Imidacloprid + Vitamin C	25.90 <sup>NS</sup> ±0.09	25.20 <sup>NS</sup> ±0.9	25.50 <sup>NS</sup> ±0.07

Data represented as mean ± stander error.

NS=Non Significant.

\*=Significant(P<0.05).

\*\*=Highly Significant(P<0.01).

Table (5): Effect of Imidacloprid and Vitamin C on liver , kidney and brain ALT and AST activities of Japanese quails at 3,6 weeks and recovery period (3 weeks ).

Parameters		Time Groups	3 weeks	6 weeks	Recovery (3 weeks)
ALT (u / g . tissue)	Liver	Control	16.50±0.15	17.70±0.54	18.40±0.16
		Vitamin C	17.10 <sup>NS</sup> ±0.24	16.70 <sup>NS</sup> ±0.42	17.20 <sup>NS</sup> ±0.42
		Imidacloprid	28.10 <sup>**</sup> ±1.45	24.70 <sup>**</sup> ±0.42	22.70 <sup>**</sup> ±0.45
		Imidacloprid + Vitamin C	20.90 <sup>*</sup> ±0.42	19.50 <sup>*</sup> ±0.35	20.50 <sup>*</sup> ±0.68
	Kidney	Control	12.70±0.68	13.70±0.54	14.40±0.42
		Vitamin C	12.30 <sup>NS</sup> ±0.29	14.92 <sup>NS</sup> ±1.89	14.90 <sup>NS</sup> ±0.89
		Imidacloprid	22.3 <sup>**</sup> ±0.29	25.80 <sup>**</sup> ±0.53	27.70 <sup>**</sup> ±1.72
		Imidacloprid + Vitamin C	15.31 <sup>*</sup> ±0.45	15.9 <sup>*</sup> ±0.76	16.41 <sup>*</sup> ±0.48
	Brain	Control	12.30±0.29	12.00±0.76	12.00±0.76
		Vitamin C	12.90 <sup>NS</sup> ±0.29	11.70 <sup>NS</sup> ±0.64	12.30 <sup>NS</sup> ±0.39
		Imidacloprid	12.60 <sup>NS</sup> ±0.29	13.62 <sup>NS</sup> ±0.29	12.40 <sup>NS</sup> ±0.42
		Imidacloprid + Vitamin C	11.90 <sup>NS</sup> ±0.42	12.40 <sup>NS</sup> ±0.42	12.90 <sup>NS</sup> ±1.89
AST (u / g . tissue)	Liver	Control	21.20±0.97	27.20±1.24	30.60±0.75
		Vitamin C	23.40 <sup>NS</sup> ±1.33	27.20 <sup>NS</sup> ±1.24	32.80 <sup>NS</sup> ±0.92
		Imidacloprid	25.40 <sup>*</sup> ±1.12	34.40 <sup>**</sup> ±1.40	34.20 <sup>*</sup> ±1.34
		Imidacloprid + Vitamin C	25.10 <sup>*</sup> ±1.32	31.9 <sup>*</sup> ±1.12	33.90 <sup>*</sup> ±0.92
	Kidney	Control	15.20±1.11	21.60±0.75	23.80±0.92
		Vitamin C	15.20 <sup>NS</sup> ±1.11	21.60 <sup>NS</sup> ±0.75	23.40 <sup>NS</sup> ±1.16
		Imidacloprid	22.20 <sup>**</sup> ±0.42	23.80 <sup>*</sup> ±0.96	26.40 <sup>*</sup> ±1.17
		Imidacloprid + Vitamin C	19.50 <sup>*</sup> ±1.11	24.10 <sup>*</sup> ±0.48	26.80 <sup>*</sup> ±0.92
	Brain	Control	18.60±1.56	22.00±0.89	24.00±0.75
		Vitamin C	19.40 <sup>NS</sup> ±0.75	23.20 <sup>NS</sup> ±0.92	25.20 <sup>NS</sup> ±0.75
		Imidacloprid	20.00 <sup>NS</sup> ±0.75	21.90 <sup>NS</sup> ±0.97	25.50 <sup>NS</sup> ±0.33
		Imidacloprid + Vitamin C	21.60 <sup>NS</sup> ±1.16	22.50 <sup>NS</sup> ±0.92	24.30 <sup>NS</sup> ±0.97

Data represented as mean ± stander error.

NS Non Significant.

\*=Significant(P&lt;0.05). \*

\*\*=Highly Significant(P&lt;0.01).



Table (6): Effect of Imidacloprid and Vitamin C on liver , kidney and brain total lipid and total cholesterol of Japanese quails at 3,6 weeks and recovery period (3 weeks ).

Parameters		Time Groups	3 weeks	6 weeks	Recovery (3 weeks)
Total lipid (mg / g . tissue)	Liver	Control	100.00±3.16	106.00±5.09	120.00±5.74
		Vitamin C	106.00 <sup>NS</sup> ±5.09	110.00 <sup>NS</sup> ±4.47	122.00 <sup>NS</sup> ±2.00
		Imidacloprid	88.00*±3.74	80.00*±6.32	99.00*±5.83
		Imidacloprid + Vitamin C	92.00 <sup>NS</sup> ±5.83	100.00 <sup>NS</sup> ±3.16	106.00 <sup>NS</sup> ±3.21
	Kidney	Control	108.00±4.47	110.00±4.00	120.00±3.47
		Vitamin C	110.00 <sup>NS</sup> ±4.06	115.00 <sup>NS</sup> ±4.74	120.00 <sup>NS</sup> ±4.47
		Imidacloprid	90.00*±3.74	92.00*±3.34	108.00*±3.16
		Imidacloprid + Vitamin C	99.00 <sup>NS</sup> ±3.33	100.00 <sup>NS</sup> ±4.47	115.00 <sup>NS</sup> ±3.45
	Brain	Control	100.00±3.16	108.00±3.16	110.00±3.40
		Vitamin C	102.00 <sup>NS</sup> ±3.46	110.00 <sup>NS</sup> ±3.45	110.00 <sup>NS</sup> ±3.16
		Imidacloprid	97.00 <sup>NS</sup> ±3.12	99.00 <sup>NS</sup> ±3.33	105.00 <sup>NS</sup> ±3.16
		Imidacloprid + Vitamin C	99.00 <sup>NS</sup> ±3.48	100.00 <sup>NS</sup> ±3.45	105.00 <sup>NS</sup> ±3.16
Total cholesterol (mg / g . tissue)	Liver	Control	50.90±2.42	54.60±2.21	56.70±0.22
		Vitamin C	51.90 <sup>NS</sup> ±2.21	56.70 <sup>NS</sup> ±2.22	54.90 <sup>NS</sup> ±0.20
		Imidacloprid	40.80*±3.45	40.50*±2.12	49.76*±0.22
		Imidacloprid + Vitamin C	45.10 <sup>NS</sup> ±2.22	48.80 <sup>NS</sup> ±2.12	52.80 <sup>NS</sup> ±2.14
	Kidney	Control	23.80±1.61	23.80±1.61	29.40±1.75
		Vitamin C	21.90 <sup>NS</sup> ±3.26	21.80 <sup>NS</sup> ±2.16	29.90 <sup>NS</sup> ±2.25
		Imidacloprid	17.90 <sup>NS</sup> ±2.01	19.80 <sup>NS</sup> ±2.23	23.90 <sup>NS</sup> ±2.20
		Imidacloprid + Vitamin C	18.00 <sup>NS</sup> ±2.15	20.50 <sup>NS</sup> ±2.01	26.50 <sup>NS</sup> ±2.16
	Brain	Control	29.40±1.75	30.10±1.23	28.50±1.23
		Vitamin C	28.90 <sup>NS</sup> ±1.26	29.90 <sup>NS</sup> ±2.25	27.50 <sup>NS</sup> ±1.20
		Imidacloprid	27.80 <sup>NS</sup> ±1.50	30.00 <sup>NS</sup> ±2.23	26.50 <sup>NS</sup> ±0.90
		Imidacloprid + Vitamin C	26.90 <sup>NS</sup> ±2.10	27.50 <sup>NS</sup> ±2.09	25.10 <sup>NS</sup> ±0.90

Data represented as mean ± stander error.

NS=Non Significant.

\*=Significant(P&lt;0.05).

\*\*=Highly Significant(P&lt;0.01).

## Discussion

The present study was planned to evaluate the biochemical changes in male Japanese quails subjected to the risk effect of imidacloprid insecticide for three and six weeks followed by a recovery period (3 weeks) after the last dose. At the same time we used vitamin C (antioxidant) as antidote to illustrate if it has any protective role against the toxicity of imidacloprid.

As well known, the liver cells are central to the intermediary metabolism of the body. Therefore, the hepatocytes have come to be considered the prototype cells in biochemistry. The liver is involved in the metabolism of most metabolites. Its primary function is the maintenance of sufficient plasma levels of these metabolites (homeostasis) (Koolman and Rohm, 1996). ALT, AST, ALP and LDH are important liver enzymes indicators used for evaluation the hepatotoxicity and liver damage in clinical investigations, these enzymes were secreted to blood in hepatocellular injuries and their levels increases (Govindwar and Dalvi, 1990). According to the obtained results, imidacloprid caused a marked significant increase in serum LDH, ALP, AST and ALT activities after quails treated with imidacloprid alone or in combination with vitamin C, then returned to the normal ranges after the recovery periods. Also, the same results of ALT and AST activities were recorded in liver and kidney tissues. In view of these data it could be assumed that imidacloprid insecticide causes a hepatic disorders, which may lead to these elevations. Therefore, the enzymes activities might reflect the damage of liver.

This elevation of enzymes activities may be caused by one of the two mechanisms, consequent to impairment of the function of liver tissues with subsequent liberation of the enzymes into the circulation from the damaged tissue or increased permeability of plasma membrane or cellular necrosis, and this showed the stress condition of the treated birds (Ignatov, 1976). Also, the increase in LDH activity may be due to the hepatocellular necrosis leading to leakage of the enzyme

into the blood stream (William and Marks, 1994). Our results are in agreement with those reported by Videla et al. (1997); Kaur et al. (2003); Rutcu et al. (2006) and Khan et al. (2008).

Evidence from biochemical studies suggest that the inhibition produced by the insecticides is due to their similarity to acetylcholine and their ability to react with the esterases in the same way as the normal substrate (Fest and Schmidt, 1982 and Shakoory et al., 1992). In this study, the obtained data revealed highly significant decrease of cholinesterase activity in groups treated with imidacloprid alone or in combination with vitamin C. This inhibition of cholinesterase activity may be due to that imidacloprid insecticide interferes with the postsynaptic receptors and it prevents the binding of acetylcholine to certain acetylcholine receptors. These results are in good agreement with those findings obtained by Westlake (1981a); Hassan et al. (1995) and Abdel-Hady & Abdeen (1997).

The present results showed that quails treated with imidacloprid with or without vitamin C exhibited a highly significant elevation in serum glucose throughout the experimental periods. Some amelioration in this elevation was detected in a recovery group treated with imidacloprid vitamin C. The elevation of glucose level can be explained by stimulation of glycogenolysis and gluconeogenesis by the liver with a temporarily loss of endocrine functions of pancreas leading to hyperglycemia (Lasram et al., 2008). Similar results were reported by Hore et al. (1997); Kalender et al. (2004) and Rezq et al. (2007).

The present results indicated that significant changes were recorded in serum total lipids and total cholesterol after treatment with imidacloprid with or without vitamin C during all experimental periods. In the same time a significant decrease of total lipid and total cholesterol was observed in liver tissues throughout the experimental periods in imidacloprid treated group. Also, a significant depletion of total lipid was detected in kidney tissue of quails treated with imidacloprid alone.

This disturbance of total lipids and total cholesterol in quail serum might have been caused by hepatic damage and the associated biliary obstruction as a result of the insecticide toxicity. The present findings receive marked support from the observations of several reports; by *Chetty et al. (1993)*; *Sadurka and Boguszewski (1993)*; *Helal et al. (1996, 1997)* and *Ismail (2005)*.

The present work indicated significant decreases in serum total protein, albumin and globulin levels in addition to the same results in liver tissue. This depletion was observed in groups treated with imidacloprid only, whereas vitamin C caused an amelioration in these depletions when combined with the insecticide.

The reduction of the protein levels might be due to disturbances of protein synthesis by the liver. This depression might be due to an alteration in intracellular protein synthesis mechanisms and changes of oxidative enzymes. These results were in agreement with those reported by *EL-Fikey et al. (1992)*; *Amer et al. (1994)* and *EL-Wardany et al. (1996)*.

Creatinine levels revealed significant increase in imidacloprid treated groups during the experimental periods, where in groups treated with imidacloprid combined with vitamin C a significant increase observed only after 3 weeks of treatment followed by no change after 6 weeks and recovery period as compared to the control quails.

The same results were found by *Ahmed (1994)* and *Gomaa (1995)*. Also, *Nandi et al. (2005)*, stated that the increase in serum creatinine in calves was an indication of impaired kidney function due to toxicity. Similarly, *Amer et al. (1994)*, and *Farid (1997)*, recorded an elevation in creatinine concentration in experimental animals treated with insecticides from different groups.

No significant changes were observed in the concentration of serum uric acid in all treated groups throughout the experimental periods. This may be due to the adaptive ability of quails to enhance the risk effect of imidacloprid insecticide on kidney product, and enhanced the glomerular filtration rate reflection (*EL-Kashoury, 1999*).

The present results revealed that imidacloprid caused many hazard effects on different organs of birds.

It is also clarify that using vitamin C antidote ameliorated many of these damages especially after the recovery period. So, it's well recommended to use vitamin C as antioxidant to prevent the toxic effect of imidacloprid.

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## دراسة فسيولوجية عن سمية المبيد الحشرى أميداكلوبريد ودور فيتامين "ج" كعامل واقى فى السمان اليابانى

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أجرى هذا البحث لدراسة المخاطر التى تتعرض لها الطيور ومنها السمان اليابانى وذلك بتعريضها إلى جرعة مخففة من المبيد الحشرى (إميداكلوبريد) والذى يعتبر واحدا من أحدث المبيدات الحشرية الشائعة الإستخدام. وفى محاولة لتفادى سمية المبيد على الطيور، فقد استخدم فيتامين "ج" كمضاد للأكسدة بإضافته مع المبيد. اشتملت التجربة على عدد 60 من ذكور السمان، قسمت إلى أربعة مجاميع متساوية (ضابطة - معاملة بفيتامين "ج" - جرعة قدرها 0.08 مللجرام/كجم من وزن الجسم - معاملة بجرعة 50/1 من الجرعة نصف الميئة للمبيد الحشرى اميداكلوبريد - معاملة بالمبيد مع فيتامين "ج") وعوملت المجموعات على فترتين 3، 6 أسابيع ، وتم ذبح خمسة من الطيور من كل مجموعة فى نهاية كل فترة تجريب، وتركزت بقية الطيور لمدة 3 أسابيع دون أى معاملة كفترة إستشفاء، وأجريت عليها جميعا القياسات الكيموحيوية سواء فى المصل أو الأعضاء المختلفة (الكبد-الكلية-المخ).

وقد أظهرت النتائج ما يلى:

- زيادة ذات دلالة إحصائية فى مستوى كل من الجلوكوز وإنزيم اللاكتيك دى هيدروجينيز والفوسفاتيز القاعدى فى السمان المعالج بالمبيد الذى استمر حتى نهاية التجربة. بينما أدت المعاملة بفيتامين "ج" إلى تحسن هذه الزيادة بعد فترة الإستشفاء.

- انخفض مستوى إنزيم الكولين استيريز بدلالة إحصائية واضحة فى كل من المجاميع المعاملة بالمبيد أو المضاف إليه فيتامين "ج" على مدار التجربة.

- سجلت مستويات الإنزيمات الناقلة للأمين ارتفاعا ذا دلالة إحصائية خاصة الألانين الناقل لمجموعة الأمين فى جميع فترات التجربة بينما أظهر مستوى نشاط إنزيم الأسبرتات الناقل لمجموعة الأمين تحسنا بعد مرور فترة الاستشفاء فى المجاميع المعاملة بالمبيد مع إضافة فيتامين "ج".



- ظهور زيادة ذات دلالة إحصائية واضحة في مستوى الدهون الكلية والكوليستيرول على مدار التجربة في المجاميع المعاملة بالمبيد أو بالمبيد مرتبطا بفيتامين "ج" ، بينما لوحظ انخفاض عال في مستوى البروتينات والألبومين والجلوبولين في المجموعات المعاملة بالمبيد فقط ، أما باقي المجموعات فلم يظهر بها أى تغير ملحوظ.

- ظل مستوى حمض اليوريك حول معدله الطبيعي بالمقارنة بالمجموعة الضابطة طوال فترات التجريب.

كما أظهرت نتائج القياسات المختلفة في الأنسجة الدلالات الآتية :-

- انخفاضا واضحا في مستوى الدهون الكلية في أنسجة الكبد والكلى على مدار التجربة في الطيور المعاملة بالمبيد بينما انخفض مستوى والكوليستيرول في نسيج الكبد في المجاميع المعاملة بالمبيد فقط.

- لوحظ انخفاض واضح في محتوى البروتين الكلى في نسيج الكبد فقط بعد مرور ثلاثة وستة أسابيع بينما لوحظ تحسن واضح بعد مرور فترة الاستشفاء في تلك المجاميع وكذلك بعد إضافة فيتامين "ج" مع المبيد. ولم يظهر تغير في باقي الأنسجة.

- أظهرت مستويات الإنزيمات الناقلة لمجموعة الأمين زيادة ملحوظة في أنسجة الكبد والكلى طوال مراحل التجربة في الطيور المعاملة بالمبيد أو مضافا إليه فيتامين "ج". بينما لم يظهر أى تغير في نسيج المخ . نستنتج مما سبق أن استخدام فيتامين "ج" يقلل كثيرا من أضرار المبيد خاصة بعد مرور فترة الإستشفاء.